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Development of Eye Colors in Drosophila: Fat Bodies and Malpighian Tubes as Sources of Diffusible Substances

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Notes:

experiment with cold Ringer extract of wild type Malpighian tubes; this extract, in *v; bw*, gave a color-value of 2.0, but in *cn bw* only about 0.8.

Finally, proof that the two tests cannot be due to the same substance, previously adduced by transplantation experiments, was given by extracting cinnabar pupae, which should contain only the v^+ substance. About 200 pupae were heated to 100°, crushed and centrifuged in nitrogen as described above, and the clear juice injected; it gave a v^+ test of about 2.5, but the *cn* test was completely negative.

Summary.—1. The substances capable of changing vermilion and cinnabar eye color toward wild type may be obtained in cell-free extracts from wild-type pupae, or the former alone from cinnabar pupae.

2. These substances are heat-stable and water-soluble, and apparently insoluble in acetone or sesame oil. They are, therefore, presumably neither proteins nor enzymes.

3. The cn^+ substance is less readily extracted than is v^+ substance.

4. Both substances are rapidly destroyed by oxidizing enzymes in the pupa juice, or, in the absence of enzymes, by dilute solutions of H_2O_2.

5. A simple method is described for roughly determining the concentration of the substances.

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DEVELOPMENT OF EYE COLORS IN DROSOPHILA: FAT BODIES AND MALPIGHIAN TUBES AS SOURCES OF DIFFUSIBLE SUBSTANCES

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From the work of Ephrussi and Beadle (see Ephrussi and Beadle[1] and Beadle and Ephrussi[2] for summaries) it is known that three diffusible substances are involved in the production of wild type eye color in *Drosophila melanogaster*. This paper is concerned with the normal sources of two of these, v^+ and cn^+ substances. Under certain conditions of genetic constitution these two substances may be produced by eye tissue itself.[1,2] Sturtevant's studies of mosaics of *D. simulans* indicated that v^+ substance

must have another normal source in the fly;[3] and it has been supposed, but not proved, that this was likewise true of cn^+ substance. Tests for the production of v^+ and cn^+ substances by a given organ can be made conveniently by transplanting the organ in question into gentically vermilion (*v*) and cinnabar (*cn*) flies, respectively. Such tests can be made in the late larval stages as shown by positive results from certain eye transplants made at this time.[1,2] In practice, tests for these substances are more sensitive if test animals carrying an eye color "dilution" gene are used. The double recessive apricot vermilion (w^a *v*) and apricot cinnabar (w^a; *cn*) have been used extensively. In many of the tests reported here, vermilion brown (*v; bw*) and cinnabar brown (*cn bw*) are used. All of these double recessive eye colors are very light; there is only, at the most, a tinge of color. A positive test is indicated by a modification in the direction of apricot or brown; the maximum change is represented by phenotypes as dark as apricot or brown. In preliminary tests, effects are roughly graded as strong, medium, weak or none: "Strong" represents a complete or nearly complete change. In certain experiments an attempt was made to use a system of numerical grades.

Fat Bodies.—Fat bodies of wild type larvae taken shortly before puparium formation (referred to as "standard" age in all tables) and transplanted to w^a *v* or *v; bw* larvae of similar age bring about a strong modification of the eye color of the host (table 1), showing that v^+ substance is released from such an implant; evidence to be mentioned later indicates that the fat body actually produces this substance. Unless otherwise specified (table 1), the greater part of one lateral fat body was transplanted, representing perhaps one-fourth to one-third of the total fat body tissue of a larva. There appeared to be no significant differences among the four possible sex-combinations of donor and recipient, and sexes are therefore disregarded in the table. In some experiments an ovary, which is embedded in the fat body, was transplanted with the fat body. This apparently did not influence the result. It will be noted that a few tests are recorded as giving negative results; these presumably represent instances of failure of the fat body to remain in the recipient. Since the fat body normally breaks down during metamorphosis, there is no way of checking this. However, when an ovary was transplanted with the fat body, dissections were made and in all flies in which the implanted ovary was found (indicating that the operation was successful), the results were positive.

In order to determine whether the production of v^+ substance is localized in any one part of the fat body, transplantations of the more or less triangular region of the fat body attached by one margin to the salivary gland (referred to in the table as "head fat") was tested (table 1). There is evidently no marked localization.

Fat bodies from cinnabar larvae produce v^+ substance, as might have

been predicted from eye-transplant experiments.[1,2] Transplants of claret fat bodies also give positive tests although here the amount of v^+ substance produced is apparently a good deal less than that produced by a comparable piece of wild type or cinnabar fat body; again this is in the result expected on the basis of previous studies.

On the assumption that production of v^+ substance by fat bodies might depend on an interaction with other tissues, transplants of wild type fat bodies, with and without ovaries, were made at earlier stages. Transplants made at approximately 21 and 40 hours before puparium formation evidently give results similar to those made at later stages (table 1).

TABLE 1

TESTS FOR THE PRODUCTION OF SUBSTANCES BY TRANSPLANTED FAT BODIES

In Some Experiments Ovaries Were Transplanted Simultaneously; Unless Queried, Success of Ovary Transplants Was Determined by Dissection of Adults

IMPLANT	HOST	HOURS BEFORE PUPARIUM FORMATION	MODIFICATION OF EYES OF HOST				TOTAL
			STRONG	MEDIUM	WEAK	NONE	
+	*w*^{a} *v*	Standard	5	9	1	0	15
+ (with ovary)	*w*^{a} *v*	Standard	3	3	0	0	6
+ (head fat)	*v; bw*	Standard	5	6	2	1	14
cn	*v; bw*	Standard	7	10	1	1	19
ca	*v; bw*	Standard	0	4	4	2	10
+	*w*^{a} *v*	21 ±	4	6	2	0	12
+	*w*^{a} *v*	40 ±	4	1	0	2	7
+ (with ovary)	*w*^{a} *v*	40 ±	4	0	0	0	4
+	*v*	Standard	0	2	0	1	3
+ (with ovary)	*v*	Standard	0	3	0	0	3
+ (with ovary?)	*v*	Standard	0	0	9	0	9
+	*cn bw*	Standard	0	0	0	27	27
+ (with ovary)	*cn bw*	Standard	0	0	0	17	17
+ (head fat)	*cn bw*	Standard	0	0	0	15	15
ca (with ovary?)	*cn bw*	Standard	0	0	0	5	5
+	*cn*	Standard	0	0	0	3	3
+ (with ovary)	*cn*	Standard	0	0	0	7	7

That the portion of fat body transplanted in these tests does not produce sufficient v^+ substance to change vermilion completely to wild type is shown by transplants of wild type fat bodies to vermilion test flies. Table 1 records the modification as "medium," which, as nearly as could be judged, was about half-way from vermilion to wild type.

In striking contrast to the tests of fat bodies for the production of v^+ substance, similar tests for the production of cn^+ substance were invariably negative (table 1). Tests were made of lateral fat bodies with and without an ovary and of "head fat" as defined above. This difference in the test for the two substances considered in conjunction with the tests of extracts reported in the accompanying paper (Thimann and Beadle[4]) constitutes strong evidence for the correctness of the conclusion previously

arrived at on other grounds that v^+ and cn^+ substances are distinct; it becomes increasingly more difficult to argue that v^+ and cn^+ substances actually represent merely different concentrations of a single substance.

Malpighian Tubes.—Transplants of wild type Malpighian tubes to *cn bw* flies at the late larval stage give strong modifications of the color of the eyes of the hosts, in many instances approaching the color of brown flies (table 2). Malpighian tubes obviously release appreciable quantities of cn^+ substance. As in the case of the release of v^+ substance by wild type fat bodies it can be shown that the amount of cn^+ substance released by four tubes (the normal number in *Drosophila*) is not sufficient to change cinna-

TABLE 2

TESTS FOR THE RELEASE OF SUBSTANCES BY MALPIGHIAN TUBES

In Tests Giving Weak Modifications, No Distinction Is Made in the Table between Dorsal and Ventral Tubes. Sexes of Donors and Hosts Are Disregarded

IMPLANT	HOST	MODIFICATION OF EYES OF HOST				TOTAL
		STRONG	MEDIUM	WEAK	NONE	
+; 4 tubes	*cn bw*	11	0	0	0	11
+; 2 ventral	*cn bw*	0	1	0	0	1
+; 4 tubes	*cn*	2	8	2	0	12
+; 2 tubes	*cn*	0	1	0	0	1
+; 1 tube	*cn*	0	0	1	0	1
+; 4 tubes	*v; bw*	19	2	0	0	21
+; 2 tubes	*v; bw*	1	1	0	0	2
cn; 2 dorsal	*v; bw*	0	0	5	1	6
cn; 2 ventral	*v; bw*	0	0	8	0	8
cn bw; 2 dorsal or ventral	*v; bw*	0	0	11	1	12
bri; 2 tubes	*v; bw*	2	0	10	1	13
bri; 2 tubes	*cn bw*	2	6	4	0	12
ca; 2 tubes	*v; bw*	0	0	18	2	20
ca; 2 tubes	*cn bw*	0	0	8	5	13
lt; 2 tubes	*cn bw*	0	0	7	1	8
ma; 2 dorsal	*cn bw*	2	5	3	0	10
ma; 2 ventral	*cn bw*	0	3	4	0	7
w; 2 tubes	*cn bw*	0	0	10	0	10

bar to wild type. The modification of the eyes of *cn* test flies recorded in table 2 as "strong" was such that the eye color appeared to be intermediate between cinnabar and wild type, but somewhat closer to wild type. This result is not surprising when it is remembered that wild type eye tissue itself produces an appreciable amount of cn^+ substance.

Similar tests for v^+ substance show that this substance, too, is released by wild type Malpighian tubes (table 2). Table 2 indicates that v^+ substance is released by *cn* and by *cn bw* Malpighian tubes, but in very definitely smaller amount than that released by wild type tubes. Malpighian tubes of bright (*bri*) larvae release appreciable quantities of both

v^+ and cn^+ substances, but probably less than the quantities released by wild type tubes. Tests of tubes of claret (*ca*), light (*lt*), maroon (*ma*) and white (*w*) larvae show (table 2) that: (1) Claret releases very small amounts of both substances; (2) Light releases a very small amount of cn^+ substance (no test was made for v^+ substance); (3) Maroon releases a moderate amount of both (less than wild type tubes); and (4) white releases a very small amount of cn^+ substance (no v^+ substance test was made).

The above tests of Malpighian tubes show an interesting and probably significant relation between color and release of v^+ and cn^+ or both substances. If we arrange these eye color mutants in a series with wild type according to depth of yellow color of the Malpighian tubes, we find that

$$+ > bw > ma \cong cn > cn\ bw \cong ca \cong lt \cong w,$$

the last four types having approximately colorless tubes. There is apparently a close relation between depth of pigmentation of the Malpighian tubes and the release of the two substances. An understanding of the significance of this apparent relation must await the result of further experiments.

According to table 2, *ca*, *lt* and *w* tubes release small amounts of cn^+ substance. If the test for cn^+ substance in *ca*, *lt* and *w* flies is made by growing *cn* eye implants in these mutants as hosts, *lt* and *w* show no evidence of having less cn^+ substance than has wild type, but *ca* gives a definitely weaker test. Evidently we are dealing here with another instance of localized difference in production of a diffusible substance.[5]

It is possible, as has been implied above, that Malpighian tubes do not produce v^+ and cn^+ substances, but rather accumulate them in the larval stage and release them later. This seems improbable since direct tests of body fluid of late larvae give no indications of the presence of v^+ substance (Ephrussi, Clancy and Beadle[6]). This argument is supported, but not definitely proved, by transplants in which wild type Malpighian tubes taken 24 hours before puparium formation were grown in older *cn bw* test animals (data included in table 3). Apparently these tests are as strong as those with older tubes.

Relation of Amount of Substance to Strength of Test.—From previous work with these diffusible substances it has seemed reasonable to assume that the amount of change produced was a function of the amount of diffusible substance limiting eye pigment development. However, direct tests of this assumption by comparing the implant-host effect of one and two eye implants gave inconclusive results.[7] Tests of the release of cn^+ substance by different numbers of Malpighian tubes are summarized in table 3. Modifications are recorded according to a numerical scheme as described in the accompanying paper (Thimann and Beadle[4]). In table 3 data are

grouped by spacing so that values within groups are strictly comparable; those between groups are less closely comparable. In the first group of tests there is a considerable amount of spread, presumably because ages were not accurately controlled, but it is obvious that two tubes give a stronger effect than one. Dorsal tubes are longer than ventral tubes and appear to give a correspondingly stronger effect. In the second and third groups, where ages were more accurately controlled, the results are more consistent. It should be noted that in the second group the tests of four tubes are not strictly comparable with those of one and two tubes because of an age difference between implant and host. These results support the

TABLE 3

COMPARISONS OF THE MODIFICATIONS BROUGHT ABOUT BY TRANSPLANTING DIFFERENT NUMBERS OF WILD TYPE MALPIGHIAN TUBES TO *cn bw* LARVAE

Sexes of Donors and Hosts Are Disregarded

IMPLANT	HOURS AFTER EGG-LAYING IMPLANT	HOST	MODIFICATION OF EYES OF HOST 5	4	3	2	1	0	MEAN GRADE	TOTAL NUMBER
1 dorsal	Standard	Same			3	3			2.5	6
2 dorsal	Standard	Same	3	3	1	0			4.3	7
1 ventral	Standard	Same	0	0	1	0	3		1.5	4
2 ventral	Standard	Same	3	1	2	0	1		3.7	7
4 tubes	Standard	Same	4	0	2				4.3	6
			MODIFICATION OF EYES OF HOST 4.3	4.0	3.7	3.3	3.0	2.7		
1 dorsal	95–98	Same			1	3	5		3.2	9
2 dorsal	95–98	Same		6					4.0	6
1 ventral	95–98	Same			2	3	3		3.3	8
2 ventral	95–98	Same		4					4.0	4
4 tubes	72–75	95–98	6						4.3	6
1 dorsal	114–117	Same				1	4	1	3.0	6
2 dorsal	114–117	Same	1	2	2				3.9	5
2 dorsal	95–98½	Same	3	1	2				4.1	6
2 ventral	95–98½	Same	1	1	1	2			3.7	5
4 tubes	72–75	96–99½	1			1			3.8	2

assumption that the modification of the eye color of the *cn bw* hosts is some function of the amount of cn^+ substance available.

Extraction of Substances from Malpighian Tubes and Fat Bodies.—As pointed out in the accompanying paper,[4] both v^+ and cn^+ substances can be extracted from wild type larval Malpighian tubes by cold or hot Ringer's solution. This is of interest in connection with the fact, mentioned above, that tests of larval body fluid are negative. Similar attempts to extract these substances from larval fat bodies gave clearly negative results, suggesting that v^+ substance is produced by fat bodies only at a later stage of development.

Tests of Other Organs.—Tests for the production of cn^+ substance by wild type salivary glands (one or two, with and without ovaries), brain tissue, gastric caeca and that portion of the hind gut including the imaginal ring (which regenerates a portion of the hind gut of the imago, Robertson[8]) have all given negative results.

Modification of Ocellus Color.—Vermilion and cinnabar flies have white or very pale ocelli while in wild type and brown flies these organs are brown. It has been noted that *cn bw* flies in which the eye color has been strongly modified by cn^+ substance have unmodified ocelli while *v; bw* flies showing a strong modification of eye color also show a strong modification of ocellus color toward brown. This suggests that ocellus color in cinnabar is autonomous in development, but that in vermilion it is not. However, it remains possible that higher concentrations of cn^+ substance will modify the ocelli of cinnabar flies.

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THE REGENERATION OF PLATE ROWS IN MNEMIOPSIS LEIDYI, AGASSIZ

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The power of Mnemiopsis to regenerate parts of its body that have been injuried in nature or that have been removed during experimentation is remarkable (Mortensen, 1913, and Coonfield, 1936 *a*). The rapidity of regeneration, the exactness reached in regulating the reformed organs, and the transparency of its body makes this animal an excellent one for experimentation. Organs such as plate rows and the apical organ can be observed clearly during their entire period of regeneration. The unusually rapid rate at which these organs reform is interpreted by me as being due either to a rapid movement and realignment of cells from the remaining parts of the removed organs or to the migration of nonspecialized cells into the wound area. This interpretation can be tested in this animal by